LIST OF U.S. CUSTOMS LABORATORY METHODS

USCL NUMBER	METHOD	TITLE
04-01	AOAC 925.21	**Preparation of Milk Sample**
04-02	AOAC 925.26	**Cream** **Preparation of Sample**
04-03	AOAC 927.03A	**Casein in Milk** **A. Method I**
04-04	AOAC 925.24	**Albumin in Milk**
04-05	AOAC 925.23	**Solids (Total) in Milk** **(IDF-ISO-AOAC Method)**
04-06	AOAC 920.107	**Solids (Total) in Cream** **(IDF-ISO-AOAC Method)**
04-07	AOAC 905.02	**Fat in Milk** **Roese-Gottlieb Method (IDF-ISO-AOAC Method)**
04-08	AOAC 989.05	**Fat in Milk** **Modified Mojonnier Ether Extraction Method**
04-09	AOAC 920.111A	**Fat in Cream** **Roese-Gottlieb Method (IDF-ISO-AOAC Method)**

USCL NUMBER	METHOD	TITLE
04-10	AOAC 977.20	Separation of Sugars in Honey Liquid Chromatographic Method
04-11	AOAC 920.105	Nitrogen (Total) in Milk Kjeldahl Method (IDF-ISO-AOAC Method)
04-12	AOAC 935.412	Sampling of Dried Milk
04-13	AOAC 932.06	Fat in Dried Milk Roese-Gottlieb Method
04-14	AOAC 945.48	Evaporated Milk (Unsweetened)
04-15	AOAC 938.05	Butter Preparation of Sample
04-16	AOAC 920.116	Moisture in Butter (IDF-ISO-AOAC Method)
04-17	AOAC 938.06B	Fat in Butter Direct Method
04-18	AOAC 925.41	Acids (Volatile) in Oils and Fats (Reichert-Meissl and Polenske values) Titrimetic Method

USCL NUMBER	METHOD	TITLE
04-19	AOAC 955.30	**Cheese** **Preparation of Samples**
04-20	AOAC 926.08	**Moisture in Cheese** **Method I**
04-21	AOAC 948.12	**Moisture in Cheese** **Method II (Rapid Screen Method)**
04-22	AOAC 933.05	**Fat in Cheese**
04-23	USCL Manual	**Differentiation of Cheese Varieties** **Made From Various Animal Species**
04-24	ASTM D 2800	**Test Method for Preparation of Methyl** **Esters from Oils for Determination of Fatty Acid Composition by Gas Chromatography**
04-25	ASTM D 1983	**Test Method for Fatty Acid Composition by** **Gas-Liquid Chromatography of Methyl Esters**
04-26	AOAC 986.13	**Quinic, Malic and Citric Acids in** **Cranberry Juice Cocktail and Apple Juice**

| USCL NUMBER | METHOD | TITLE |

Analysis of Buffalo and Bovine Mozzarella Cheese Mixtures by Polyacrylamide Gel Isoelectric Focusing

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-01 Index

AOAC 925.21
Preparation of Milk Sample

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States. With these types of commodities, the sample must be prepared before analysis can occur. This method is recommended when applicable.

2 REFERENCES

AOAC 925.21
Preparation of Milk Sample

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-02 Index

AOAC 925.26
Cream
Preparation of Sample

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States. With these types of commodities, the sample must be prepared before analysis can occur. This method is recommended when applicable.

2 REFERENCES

AOAC 925.26
Cream
Preparation of Sample

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-03 Index

AOAC 927.03A
Casein in Milk
A. Method I

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). Note that casein is also provided for in Chapter 35 HTSUS. **This method is recommended for the determination of casein.**

2 REFERENCES

AOAC 927.03A
Casein in Milk
A. Method I

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-04 Index

AOAC 925.24
Albumin in Milk

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). Note that albumin is also provided for in Chapter 35 HTSUS. **This method is recommended for the determination of albumin.**

2 REFERENCES

AOAC 925.24
Albumin in Milk

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-05 Index

AOAC 925.23
Solids (Total) in Milk

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is recommended for the determination of total solids in milk.**

2 REFERENCES

AOAC 925.23

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-06 Index

AOAC 920.107
Solids (Total) in Cream

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is recommended for the determination of total solids in cream.**

2 REFERENCES

AOAC 920.107
Solids (Total) in Cream
(IDF-ISO-AOAC Method)

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-07 Index

AOAC 905.02
Fat in Milk
Roese-Gottlieb Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is one of the methods recommended for the determination of fat in milk.**

2 REFERENCES

AOAC 905.02
Fat in Milk
Roese-Gottlieb Method
(IDF-ISO-AOAC Method)

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-08 Index

AOAC 989.05
Fat in Milk
Modified Mojonnier Ether Extraction Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). This method is one of the methods recommended for the determination of fat in milk.

2 REFERENCES

AOAC 989.05
Fat in Milk
Modified Mojonnier Ether Extraction Method

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-09 Index

AOAC 920.111A
Fat in Cream
Roese-Gottlieb Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is recommended for the determination of fat in cream.**

2 REFERENCES

AOAC 920.111A
Fat in Cream
Roese-Gottlieb Method
(IDF-ISO-AOAC Method)

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-10 Index

AOAC 977.20
Separation of Sugars in Honey
Liquid Chromatographic Method

AOAC 977.20
Separation of Sugars in Honey
Liquid Chromatographic Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

The separation and identification of individual sugars in different commodities are important in determining the classification within the Harmonized Tariff System of the United States (HTSUS). Although not specifically designed for wide application, the general procedure described in this method is applicable to different commodities if the sample is properly prepared. The column may be replaced with a silica-based amino (NH_2) column or equivalent.

This method has been used when determining the sugars in commodities ranging from honey to dairy products to chocolate.

2 REFERENCES

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-11 Index

AOAC 920.105
Nitrogen (Total) in Milk
Kjeldahl Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method recommended for the determination of total nitrogen in milk.**

2 REFERENCES

AOAC 920.105
Nitrogen (Total) in Milk
Kjeldahl Method
(IDF-ISO-AOAC Method)

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-12 Index

AOAC 935.412
Sampling of Dried Milk

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States. This method is recommended to sample dried milk.

2 REFERENCES

AOAC 935.412
Sampling of Dried Milk

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-13 Index

AOAC 932.06
Fat in Dried Milk
Roese-Gottlieb Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is recommended for the determination of fat in dried milk.**

2 REFERENCES

AOAC 932.06
Fat in Dried Milk
Roese-Gottlieb Method

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-14 Index

AOAC 945.48
Evaporated Milk (Unsweetened)

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States. This method is recommended for use with unsweetened evaporated milk.

2 REFERENCES

AOAC 925.21
Preparation of Milk Sample

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-15 Index

AOAC 938.05
Butter
Preparation of Sample

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States. Butter must be prepared before analysis can occur. This method is recommended when applicable.

2 REFERENCES

AOAC 938.05
Butter
Preparation of Sample

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-16 Index

AOAC 920.116
Moisture in Butter

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is recommended for the determination of moisture in butter.**

2 REFERENCES

AOAC 920.116
Moisture in Butter
(IDF-ISO-AOAC Method)

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-17 Index

AOAC 938.06B
Fat in Butter
Direct Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Milk and milk products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is recommended for the determination of fat in butter.**

2 REFERENCES

AOAC 938.06B
Fat in Butter
Direct Method

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-18 Index

AOAC 925.41
Acids (Volatile) in Oils and Fats
(Reichert-Meissl and Polenske Values)
Titrimetric Method

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Certain high fat containing dairy products are provided for in Chapter 4 and Chapter 21of the Harmonized Tariff Schedule of the United States (HTSUS). **This method is one that can be used for the determination of volatile acids in oils and fats.**

2 REFERENCES

AOAC 925.41
Acids (Volatile) in Oils and Fats
(Reichert-Meissl and Polenske Values)
Titrimetic Method

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-19 Index

AOAC 955.30
Cheese
Preparation of Sample

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Cheese and related products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States. With these types of commodities, the sample must be prepared before analysis can occur. This method is recommended when applicable.

2 REFERENCES

AOAC 955.30
Cheese
Preparation of Sample

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-20 Index

AOAC 926.08
Moisture in Cheese
Method I

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Cheese and related products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS).
This method is recommended for the determination of moisture in cheese.

2 REFERENCES

AOAC 926.08
Moisture in Cheese
Method I

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-21 Index

AOAC 948.12
Moisture in Cheese
Method II

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Cheese and related products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS).
This method is a screening method for the determination of moisture in cheese.

2 REFERENCES

AOAC 948.12
Moisture in Cheese
Method II
(Rapid Screen Method)

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-22

Index

AOAC 933.05
Fat in Cheese

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Cheese and related products are provided for in Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS). This method is recommended for the determination of fat in cheese.

2 REFERENCES

AOAC 933.05
Fat in Cheese

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-23 INDEX

Differentiation Cheese Varieties Made From Various Animal Species

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

0 INTRODUCTION

Cheese made wholly or in part from cows' milk are subject to import quota restrictions. It is necessary to have analytical procedures to differentiate from the animal origin of cheeses for this reason. The following describes a screening method for this purpose.

1 SCOPE AND FIELD OF APPLICATION

This method is used as a screening procedure to differentiate cheeses made solely from cows' milk or from other animal species such as sheep, goat, water buffalo or from a mix of different species. However, for cheese made from a mixture of different species, the results must be interpreted carefully. Other methods such as electrophoresis or capillary electrophoresis may be employed to determine the percentage of different varieties in the mixture.

2 REFERENCES

Differential Characteristics of Fatty Acids in Cheese from Milk of Various Animal Species by Capillary Gas Chromatography, Prager, M.J., in ***J. Assoc. Off. Anal. Chem.*, 1989 ,** 72(3), 418-421

3 REAGENTS AND APPARATUS

3.1 GC Column is a 30 m x 0.25 mm id SP-2340 (Supelco, Inc., Bellefonte, PA 16923) fused silica column or equivalent, film thickness 0.20 µm

3.2 Gas Chromatograph is an HP 5890 (Hewlett-Packard) equipped with a flame ionization detector (FID) or equivalent GC with attached integrator

3.3 Operation Parameters

3.3.1 Initial temperature of 30EC; hold time of 4 minutes then ramp at 4EC/min until 250EC; hold for 10 minutes

3.3.2 Injector temperature is 250EC

3.3.3 Detector temperature is 275EC

3.3.4 Helium gas at 30 mL/min; hydrogen gas at 30 mL/min; air at 300 mL/min

3.4 Tetramethylammonium hydroxide (Stock solution: 25 mL of 2 M in methanol)

3.5 GC Standards are fatty acid methyl esters mixture (Alltech Associates, Inc., Deerfield IL 60015)

3.6 Dichloromethane, reagent grade

3.7 Blender

3.8 Heater (heating block)

4 SAMPLE PREPARATION

4.1 Mix five grams of cheese with 100 mL of dichloromethane for 30 seconds in a blender.

4.2 Filter mixture under suction into a filter flask containing a small amount of anhydrous sodium sulfate.

4.3 Decant filtrate, filter the mixture under suction into a filter flask containing a small amount of anhydrous sodium sulfate.

4.4 Decant filtrate into evaporating dish and evaporate almost to dryness on a steam bath.

4.5 Transfer 10 µL of residue to a 2 mL-crimp-top vial and 1 mL of 2 M tetramethylammonium hydroxide in methanol. Heat the sealed vial on a heating block until the contents are completely dissolved.

5 PROCEDURE

5.1 Establish the retention times of individual fatty acid ester by injecting 0.2 µL of the GC standard of fatty acid esters mixture.

5.2 Inject 0.2 µL solution of prepared cheese sample onto the column.

5.3 Acquire peak area percentages for major components and directly calculate all possible independent ratios.

6 RESULTS

6.1 Calculate and compare all possible rations of different fatty acids. Interpret the results according to Tables 1, 2 or 3.

6.2 For cheeses made from a single species, most of all fatty acid rations should fall in the range. It may be necessary to rerun the fatty acid profiles if there is any doubt of data out of this range.

7 LIMITATIONS

It is recommended to have triplicate runs to calculate the average peak areas of individual fatty acid esters. The method is used as a screening process to differentiate the animal origin of cheeses. The results may be further confirmed by electrophoresis, capillary electrophoresis, or immunoassay.

Table 1. Fatty acid peak area response ranges and averages

	Cheeses made with milk from							
	Cows		Buffalo		Sheep		Goats	
Acid	Range, %	Av., %	Range, %	Av., %	Range, %	Av., %	Range, %	Av., %
C8:0	0.72–1.40	1.04	0.57–1.05	0.79	1.21–2.72	1.94	2.07–2.78	2.32
C10:0	2.20–3.37	2.80	1.24–2.38	1.66	3.76–8.20	6.01	7.53–9.89	8.61
C12:0	2.63–4.22	3.30	1.39–3.08	2.18	2.53–5.34	3.67	3.48–4.77	4.16
C14:0	9.31–13.3	10.6	8.41–12.4	9.96	6.57–12.5	9.47	8.09–11.6	9.94

Table 2. Fatty acid peak area response ratio ranges and averages

	Cheese samples made with milk from							
	Cows		Buffalo		Sheep		Goats	
Ratio	Range, %	Av., %	Range, %	Av., %	Range, %	Av., %	Range, %	Av., %
C10:0/C8:0	2.05–3.10	2.73	1.77–2.35	2.07	2.56–3.89	3.23	3.26–4.15	3.72
C12:0/C8:0	2.85–4.22	3.17	2.43–3.46	2.76	1.44–2.73	1.94	1.47–2.24	1.80
C14:0/C8:0	7.68–12.5	9.93	10.5–19.7	13.3	3.48–6.86	5.12	3.15–5.12	4.31
C16:0/C8:0	19.7–37.9	27.2	25.7–55.4	37.3	6.81–18.0	12.1	8.49–13.0	11.1
C18:0/C8:0	5.48–14.9	10.4	12.4–21.9	17.0	3.05–9.45	5.70	3.09–5.50	4.27
C18:1/C8:0	15.3–33.1	24.2	23.6–43.5	32.0	8.38–20.4	12.9	7.95–10.9	9.49
C12:0/C10:0	1.02–1.40	1.18	1.09–1.51	1.32	0.52–0.73	0.61	0.44–0.57	0.48
C14:0/C10:0	3.10–4.67	3.84	5.02–7.17	6.06	1.18–2.23	1.62	0.94–1.31	1.16
C16:0/C10:0	7.66–13.1	10.0	13.0–23.9	18.2	2.42–5.85	3.78	2.58–3.72	3.03
C18:0/C10:0	2.46–5.08	3.79	6.39–10.9	8.21	0.88–3.11	1.85	0.83–1.67	1.16
C18:1/C10:0	5.96–12.2	8.99	11.1–20.0	15.4	2.55–5.72	4.07	1.95–3.19	2.56
C14:0/C12:0	2.62–4.03	3.26	3.94–5.33	4.55	2.16–3.58	2.64	2.14–2.75	2.39
C16:0/C12:0	6.10–10.7	8.48	9.55–17.7	12.9	4.16–9.39	6.13	5.28–8.16	6.29
C18:0/C12:0	1.93–4.37	3.23	3.62–9.33	6.01	1.33–4.93	3.03	1.68–3.52	2.41
C18:1/C12:0	5.61–10.6	7.87	8.06–15.1	11.5	3.49–9.32	6.69	3.88–6.70	5.39
C16:0/C14:0	2.15–3.00	2.60	2.09–3.59	2.86	1.82–2.76	2.30	2.37–2.78	2.57
C18:0/C14:0	0.56–1.34	1.01	0.73–1.81	1.26	0.55–1.70	1.14	0.71–1.44	1.01
C18:1/C14:0	1.67–3.09	2.36	1.60–3.69	2.48	1.50–3.62	2.57	1.64–3.10	2.23
C18:0/C16:0	0.19–0.52	0.39	0.26–0.87	0.47	0.24–0.64	0.49	0.24–0.56	0.39
C18:1/C16:0	0.66–1.27	0.91	0.58–1.27	0.87	0.64–1.52	1.13	0.66–1.13	0.85
C18:1/C18.0	1.26–3.10	2.31	1.46–2.69	2.01	1.65–3.02	2.31	1.90–2.57	2.20

Table 3. Fatty acid ratios for cheese found to contain cow's milk

	Cheese samples purported to be made with milk from								
	Buffalo			Sheep			Goats		
Ratio	1	2	3	4	5	6	7	8	9
C10:0/C8:0	2.44	2.42	2.80	5.71	2.42	2.35	2.35	2.50	2.47
C12:0/C8:0	2.69	2.77	3.31	4.15	3.67	2.75	2.78	3.03	1.67
C14:0/C8:0	9.63	6.63	13.1	9.33	8.78	9.58	9.62	10.4	5.71
C16:0/C8:0	27.8	26.9	36.3	23.5	19.8	27.4	28.3	30.3	13.6
C18:0/C8:0	10.9	8.19	12.8	4.88	8.09	5.83	8.71	6.52	10.1
C18:1/C8:0	26.4	21.1	29.6	20.7	17.7	18.1	22.5	18.7	18.3
C12:0/C10:0	1.10	1.15	1.18	0.73	1.52	1.17	1.18	0.86	0.67
C14:0/C10:0	3.94	2.74	4.67	1.63	3.63	4.08	4.09	4.17	2.31
C16:0/C10:0	11.4	11.1	13.0	4.10	8.20	11.7	12.0	12.1	5.49
C18:0/C10:0	4.46	3.39	4.58	0.85	3.34	2.48	3.70	2.60	4.08
C18:1/C10:0	10.8	8.74	10.6	3.62	7.32	7.70	9.55	7.47	7.42
C14:0/C12:0	3.58	2.39	3.96	2.25	2.39	3.48	3.46	3.45	3.43
C16:0/C12:0	10.4	9.69	11.0	5.65	5.41	9.97	10.2	10.0	8.13
C18:0/C12:0	4.05	2.95	3.88	1.18	2.20	2.12	3.13	2.16	6.04
C18:1/C12:0	9.82	7.61	8.96	4.98	4.82	6.58	8.08	6.18	11.0

ASTM D 2800
Test Method for Preparation of Methyl Esters from Oils for Determination of Fatty Acid Composition by Gas Chromatography

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Oils from many different sources can be characterized by looking at their fatty acid composition. In order to use gas chromatography, the fatty acids are converted into their corresponding methyl esters. This method describes a method for this reaction.

Since oils and related products are classified in many different Chapters of the Harmonized Tariff Schedule of the United States (HTSUS), this method has wide application.

2 REFERENCES

ASTM D 2800
Test Method for Preparation of Methyl Esters from Oils for Determination of Fatty Acid Composition by Gas Chromatography

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-25 Index

ASTM D 1983
Test Method for Fatty Acid Composition by Gas Chromatography of Methyl Esters

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Oils from many different sources can be characterized by looking at their fatty acid composition. In order to use gas chromatography, the fatty acids are converted into their corresponding methyl esters. This method describes the gas chromatographic methodology.

Since oils and related products are classified in many different Chapters of the Harmonized Tariff Schedule of the United States (HTSUS), this method has wide application.

2 REFERENCES

ASTM D 1983
Test Method for Fatty Acid Composition by Gas-LIquid Chromatography of Methyl Esters

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-26 Index

AOAC 986.13
Qunic, Malic and Citric Acids in Cranberry Juice and Apple Juice

SAFETY PRECAUTIONS

This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to its use.

1 SCOPE AND FIELD OF APPLICATION

Organic acids often characterize juices and other foods, including oils. This method uses a liquid chromatographic technique to identify the organic acids. Because of the range of commodities that can be analyzed using this method, it has wide applicablitiy to commodities in the Harmonized Tariff Schedule of the United States.

2 REFERENCES

AOAC 986.13
Quinic, Malic and Citric Acids in Cranberrry Juice Cocktail and Apple Juice

U.S. CUSTOMS LABORATORY METHODS

USCL METHOD 04-27

INDEX

Analysis of Buffalo and Bovine Mozzarella Cheese Mixtures by Polyacrylamide Gel Isoelectric Focusing

SAFETY PRECAUTION

This method does not purport to address all the safety problems, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

0 SCOPE AND FIELD OF APPLICATION

This separation technique utilizes a dry isoelectric focusing (IEF) PhastGel with the PhastSystem apparatus (Pharmacia-LKB Biotechnology) to distinguish mozzarella cheese derived from water buffalo milk and bovine milk. The technique is useful for establishing the authenticity of a water buffalo cheese and allows semiquantitative determination of mixtures of the two animal sources.

1 PRINCIPLE

The base-soluble proteins are concentrated and isolated from the cheese matrix. Treatment with the enzyme plasmin converts the beta caseins to gamma caseins, greatly enhancing their naturally low concentration. The gamma caseins of the two species have different isoelectric points which allow the differentiation to be made by the IEF gel electrophoresis procedure. By preparing known mixtures of the dried isolated proteins, estimation of bovine milk content in water buffalo cheese can be achieved to as low as 2% (*w/w*).

2 REFERENCES

Trieu-Cuot, P., Gripon C., *J. Dairy Research* (1981) **48**, 303.

Trieu-Cuot, P., Addeo F., *J. Dairy Research* (1981) **48**, 311.

Addeo, F. Moiio L., Chianese L. and Nota G. *Ital J. Food Sci.* (1989) **3**, 71.

Moio, L., Chianese L., Addeo F., *Pharmacia Applic. Note 374.*

3 METHOD

3.1 Apparatus

3.1.1 PhastSystem apparatus (Pharmacia - LKB Biotechnology)

3.1.2 Dry gel reconstituting cassette

3.1.3 Sample applicators [(8/1); 8 lanes/1µL delivered]

3.1.4 Dry IEF gels (Pharmacia).

3.1.5 Centrifuge capable of spinning at

3000 RPM

3.1.6 Analytical balance (± 0.0001 g)

3.1.7 Mortar and pestle

3.1.8 Vortex mixer

3.1.9 Screw-capped culture tubes (16 mm x 100 mm, 16mm x 125 mm)

3.1.10 37EC water bath

3.1.11 Erlenmeyer flasks (125 mL or larger)

3.1.12 Pasteur pipets

3.1.13 Magnetic stirrer

3.1.14 Automatic pipets covering 1-1000 µL

3.1.15 Disposable pipet tips covering 1-1000 µL

3.1.15 Micro centrifuge tubes

3.1.16 Syringe, approximately 3 cc capacity

3.2 Reagents, Materials and Solutions

3.2.1 Reagents

3.2.1.1 Deionized water (18 megohm-cm minimum purity)

3.2.1.2 Plasmin Suspension (Boehringer Mannheim, bovine origin)

3.2.1.3 Trichloroacetic acid (TCA), ACS reagent

3.2.1.4 Sodium tetraborate, ACS Reagent

3.2.1.5 Ampholine, pH 5-7 (Pharmacia)

3.2.1.6 Pharmalyte, pH 6.7-7.7 (Pharmacia)

3.2.1.7 Urea, ACS Reagent

3.2.1.8 Glacial acetic acid, ACS Reagent

3.2.1.9 95% *(v/v)* Ethanol, ACS Reagent

3.2.1.10 Petroleum Ether, ACS Reagent, bp 36-60EC

3.2.2 Materials and Solutions

3.2.2.1 pH Indicator papers, pH range 0 - 14

3.2.2.2 Hydrophilic membrane filters (0.45 µm pores, nylon or cellulose acetate membrane), syringe-style

3.2.2.3 50 mM sodium tetraborate buffer:
Add 10.06 g of granular anhydrous sodium tetraborate or 19.07 g of sodium tetraborate decahydrate to 1000 mL of deionized water. Stir to dissolve. The solution pH should be between 9.0 - 9.5.

3.2.2.4 8 M urea-borate buffer:
Dissolve 9.600 grams urea and 0.2012 grams sodium tetraborate in sufficient deionized water in a graduated Erlenmeyer flask to obtain 20 mL solution. This solution should be prepared daily as needed.

3.2.2.5 7.8 M urea:
Dissolve 4.68 grams urea in sufficient deionized water in a graduated Erlenmeyer flask to obtain 10 mL solution. This solution should be prepared daily as needed.

3.2.2.6 24% (*w/w*) TCA solution:
Add 12 g of TCA to 38 mL

deionized water in a 125 mL Erlenmeyer flask.

3.2.2.7 20% (w/w) TCA solution: Add 10 g of TCA to 40 mL deionized water in a 125 mL Erlenmeyer flask.

3.2.2.8 1:1 (v/v) Petroleum Ether/Water: Mix equal volumes of petroleum ether and deionized water.

3.2.2.9 PhastGel Silver Kit, #17-0617-01 (Pharmacia)

3.2.2.10 Rehydration solution for the PhastGel dry IEF: Prepare a solution by mixing 3.6 mL of 7.8 M urea, 0.2 mL Ampholine pH 5-7, and 0.2 mL of Pharmalyte pH 6.7-7.7.

3.3 References

3.3.1 Fresh mozzarella cheese (water buffalo) from a known source

3.3.2 Fresh mozzarella cheese (bovine) from a known source

4 SAMPLING

4.1 A fresh cheese sample should be obtained and kept refrigerated. The sample should be frozen, if the sample cannot be analyzed within 2 weeks. Samples that are stored for long periods of time should always be stored in a frozen state.

5 PROCEDURE

5.1 Weigh a 1-2 gram sample of cheese to ±0.01 g.

5.2 Homogenize the sample in 10.0 mL of 50 mM sodium tetraborate buffer (pH 9.0-9.5). Transfer the solution to a screw cap culture tube (16 mm x 125 mm).

5.3 Vortex the solution and allow it to stand 5 minutes.

5.4 Centrifuge the sample at 3000 RPM (minimum) at room temperature for 10 minutes.

5.5 Transfer 1.5 mL of the fat-free portion of the supernatant solution to a preweighed culture tube (16 mm x 100 mm).

5.6 Add 25 µL (or sufficient volume to supply 0.125 Units) of plasmin (Boehringer Mannheim bovine origin) .

5.7 Incubate for 1 hr at 37EC in a water bath.

5.8 Precipitate the proteins by adding 1.5 mL of 24% (*m/m*) TCA and vortex for 30 seconds.

5.9 Centrifuge at 3000 RPM (minimum) for 10 minutes at room temperature. Discard the supernatant.

5.10 Wash the precipitate with 10 mL of a 1:1 (v/v) petroleum ether/deionized water solution. Wash the precipitate again with 10 mL of deionized water. Mixing is achieved by vortexing for 30 seconds. Centrifuge at 3000 RPM (minimum) for 10 minutes at room temperature. Discard the supernatant after each wash.

5.11 For a qualitative species determination, this precipitate, the wet weight sample, can be analyzed by proceeding to **5.14**.

5.12 If the analysis is to continue on the next day, invert the tube in a tube rack and allow the precipitate, the protein pellet, to air dry overnight. This provides the dry weight sample,

which is suitable for a semiquantitative determination.

5.13 Reweigh the test tube with the sample in it and calculate the weight of the protein sample.

5.14 Solubilize the sample with sufficient volume of 8 M urea-borate buffer solution to achieve a final concentration of 2% (v/v) for the dry weight sample or 10% (*v/v*) for the wet weight sample. If the sample is turbid, filter through a small pore (0.45 µm) hydrophillic membrane filter.

5.15 Rehydrate the PhastGel dry IEF in a PhastGel cassette according to the instructions provided with the cassette kit and Pharmacia Application Note 374.

5.16 Apply 1 µL of the sample to the anode side of the gel according to the procedure in the PhastSystem user manual using the PhastGel 8/1 applicator.

5.17 The electrophoresis program for the separation of the proteins is as follows:

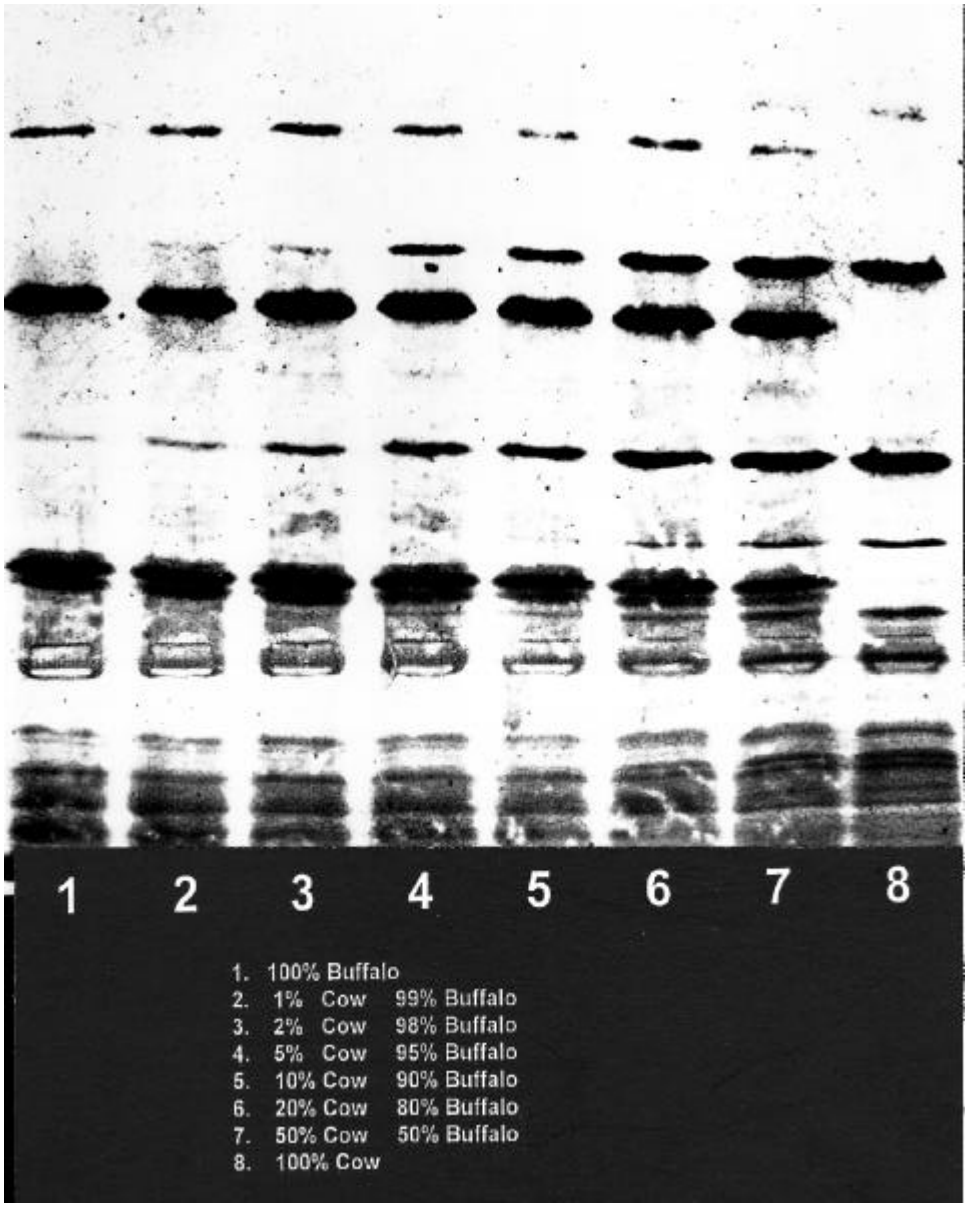
Sample Applicator down at			1.2		0 Vh
Sample Applicator up at 1.2			15 Vh		
Sep 1.1	1200V	2 mA	2.5W	15C	75 Vh
Sep 1.2	200V	2 mA	2.5W	15C	15 Vh
Sep 1.3	1200V	5 mA	2.5W	15C	710 Vh

5.18 The gel is stained and fixed according to the directions enclosed with the PhastGel Silver Staining Kit.

5.19 The gel should either be mounted in plastic or a picture should be taken of the gel for the permanent record.

5 RESULTS

An example of the type of results that can be obtained are in Figure 1. The semiquantitative determination for a cheese prepared from a mixture of the two milk sources requires comparison to appropriate mixtures of solutions of the pure proteins similarly isolated from authentic bovine and water buffalo milk cheeses.


1 2 3 4 5 6 7 8
1. 100% Buffalo
2. 1% Cow 99% Buffalo
3. 2% Cow 98% Buffalo
4. 5% Cow 95% Buffalo
5. 10% Cow 90% Buffalo
6. 20% Cow 80% Buffalo
7. 50% Cow 50% Buffalo
8. 100% Cow